CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-618

21-681

21-682

PHARMACOLOGY REVIEW

CDER STANDARD COVERSHEET

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA's:	21-618; 21-681; 21-682
Product Name:	[redacted] (Tinidazole)
Sponsor:	Presutti Laboratories
Indications:	Trichomoniasis (NDA 21-618) Giardiasis (NDA 21-681) Amebiasis (NDA 21-682)
Division:	Special Pathogen and Immunologic Drug Products HFD-590
Reviewer:	Stephen Hundley, Ph.D., DABT Acting Pharmacology/Toxicology Team Leader, HFD-590
Date:	4/2/04

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1.0 EXECUTIVE SUMMARY

1.1 Recommendations

Recommendations on Approvability:

The sponsor submitted tinidazole under 505 (b) (2) designation and supported nonclinical and clinical safety requirements for the proposed indications with published scientific literature. The sponsor also conducted and submitted a reproductive toxicology study that evaluated the effect of tinidazole on male rat fertility. The information from the nonclinical pharmacology and toxicology literature articles and results from the rat fertility study supported approving the following proposed indications and dosing regimens from the nonclinical safety perspective:

- Trichomoniasis: 1) single 2 g oral dose (one day) with food; [redacted]
- Giardiasis: 1) single 2 g oral dose (one day) with food; 2) [redacted] 3) single 50 mg/kg oral dose to pediatric patients over the age of 3 years (one day) not to exceed 2 g.
- Amebiasis: 1) single 2 g oral dose for 3 consecutive days for intestinal amebiasis; 2) 2 g daily oral dose for 3 to 5 days for amebic liver abscess; 3) single 50 mg/kg oral dose for 3 days to pediatric patients over the age of 3 for intestinal amebiasis not to exceed 2 g daily.

Recommendation for Additional Nonclinical Studies:

The Pharmacology/Toxicology Reviewer requested, in a Teleconference on 3/12/02, that the sponsor conduct a one-month oral toxicity study with tinidazole in either dogs or monkeys as a post-marketing commitment. The sponsor subsequently submitted a protocol outline for a 30-day toxicity study in beagle dogs that will be initiated following the final decision on the NDA application.

Recommendations on Labelling:

The sponsor was informed in discussions prior to the submission of the NDA that the final tinidazole label will include a boxed warning similar to metronidazole (Flagyl®) with regard to carcinogenicity in rodents with the nitroimidazole class of compounds. Labelling information in the "Carcinogenesis, Mutagenesis, Impairment of Fertility" and the "Pregnancy:Teratogenic Effects" sections should include the appropriate information from the literature cited by the sponsor. The genotoxicity and mutagenicity information should specifically identify the test system and results (positive or negative for

genotoxicity/mutagenicity). Dose levels for the developmental toxicity studies should be correlated to the maximum human dose based upon body surface area conversions or area under the plasma concentration vs time curve (AUC) data. Tinidazole is not an approved drug product in the United States; consequently there is no FDA-approved label.

1.2 Summary of Nonclinical Findings

Pharmacologic Activity

Literature information regarding the antiprotozoal activity of the nitroimidazoles in general and tinidazole in particular were examined by the Clinical and Microbiology Reviewers. Therefore, the nonclinical pharmacologic activity of tinidazole was not addressed in the Pharmacology/Toxicology Review and Evaluation.

Nonclinical Overview

Single-dose oral toxicity studies with tinidazole generated LD_{50} values of approximately 3,000 mg/kg for rats and 4,000 mg/kg for mice. Tinidazole dose levels in a one-month study with rats ranged from 125 to 4,000 mg/kg/day. Most of the animals at the 4,000 mg/kg/day level died and mortality (2 of 15 females) was also observed at the 2,000 mg/kg/day dose level. No mortality or clinical signs of toxicity were observed at dose levels of 1,000 mg/kg/day and lower. Gross pathology indicated elevated liver weights and reduced testes and epididymis weights at the 1,000 and 2,000 mg/kg/day dose levels. Histopathological observations at the 1,000 and 2,000 mg/kg/day dose levels included pleomorphism in the liver, atrophy of seminiferous tubules, inhibition of spermatogenesis, and absence of spermatogoniums in the seminiferous tubules of the testes. No compound-related histopathological effects were observed at dose levels of 500 mg/kg/day and lower. The no-observed adverse-effect level (NOAEL) for the one-month study was 500 mg/kg.

A six-month oral toxicity study in rats generated no compound-related effects at the 60 and 150 mg/kg/day dose levels. The 600 mg/kg/day dose level resulted in elevated liver weights, reduced testes and epididymis weights, and testicular atrophy. Mild to moderate degeneration of seminiferous tubules in the testes was observed at the 600 mg/kg/day dose level. Histopathology of the liver revealed pleomorphism of hepatocytes and slight disarray of hepatic cell clusters at both the 300 and 600 mg/kg/day dose levels The NOAEL for this study was 150 mg/kg.

Embryo-fetal developmental toxicity studies in pregnant rats and mice resulted in no maternal toxicity to mice at the highest dose level of 2,500 mg/kg/day; maternal toxicity was not observed in pregnant rats at a dose level of 500 mg/kg/day. Maternal mortality resulted from the 2,000 mg/kg/day dose level to pregnant rats. No embryo-fetal toxicity or developmental abnormalites were observed in mice at the highest dose level of 2,500 mg/kg/day. An elevated incidence of embryo-fetal mortality was observed in rats at the 500 and 2,000 mg/kg/day dose levels while fetal skeletal development was delayed at the

2,000 mg/kg/day dose level. Fetal malformtations were not observed in rats at any maternal dose level. NOAEL's were 2,500 mg/kg for maternal and embryo-fetal effects in mice, 500 mg/kg for maternal toxicity in pregnant rats, and 125 mg/kg for embryo-fetal toxicity and fetal development in rats.

The maturation and development of neonatal rats and mice were monitored for a period of six weeks following birth. No developmental effects were observed at any maternal tinidazole dose level. The NOAEL's were 2,000 and 2,500 mg/kg for rats and mice, respectively. In a separate developmental toxicity study in pregnant rats there was no evidence of maternal toxicity at the highest tinidazole dose level of 600 mg/kg/day (oral gavage dosing on Days 1 through 21 of gestation). A marginally higher incidence of fetal mortality was observed at the 600 mg/kg/day dose level. Skeletal or visceral malformations were not observed in the offspring at the 2-month post-partum sacrifice. Slightly depressed responsiveness was briefly observed in neonates (0 to 24 hours post-partum) at the 600 mg/kg/day maternal dose level. The NOAEL for fetal toxicity was 300 mg/kg. The NOAEL for maternal toxicity was 600 mg/kg.

Tinidazole reduced male rat fertility at a daily oral dose level of 600 mg/kg/day for 30 days. Spermatogenic effects and testicular histopathology were observed at the 300 and 600 mg/kg/day dose levels. The testicular histopathological effects at the 600 mg/kg/day dose level were classified as severe. The NOAEL for testicular effects was 100 mg/kg/day.

The mutagenicity of tinidazole was evaluated in the *Salmonella typhimurium* mutagenicity test in the presence and absence of a rat hepatic S-9 metabolic activation system. Tinidazole was consistently mutagenic in the TA 100, *S. typhimurium* tester strain both with and without the metabolic activation system and was negative for mutagenicity in the TA 98 strain. Mutagenicity results were mixed in the TA 1535, 1537, and 1538 strains. Tinidazole was mutagenic in a tester strain of *Klebsiella pnuemonia.* Tinidazole was negative for mutagenicity in a mammalian cell culture system utilizing Chinese hamster lung V79 cells (HGPRT test system) and negative for genotoxicity in the Chinese hamster ovary (CHO) sister chromatid exchange assay. Tinidazole induced elevated numbers of sister chromatid exhanges in freshly isolated human lymphoctye cell cultures. Tinidazole was postive for *in vivo* genotoxicity in the mouse micronucleus assay.

Tinidazole metabolism studies in rats and dogs indicated that the primary route of excretion was in the urine (60 to 70 percent) with the remainder in feces. Plasma half-lives were approximately 8 to 10 hours in dogs. Unchanged tinidazole was the major urinary excretion product in rats and dogs. Structurally characterized metabolites included ring methyl group hydroxylation and a glucuronide to this hydroxylated product. Tissue distributition data for radioactivity derived from radiolabelled tinidazole indicated no evidence for accumulation in any organ or tissue.

Nonclinical Safety Issues

Clinical safety experience with tinidazole as an approved drug product in Europe for the past 30 years supersedes most potential nonclinical safety issues. The only issue that needs to be addressed is the examination of repeat-dose toxicity in a nonrodent species. The sponsor submitted an acceptable protocol for a 30-day repeat-dose oral toxicity study in beagle dogs as a post-marketing commitment. In addition to providing organ-specific toxicity information in a nonrodent species, this study should determine the extent to which tinidazole generates testicular toxicity in male dogs compared to male rats.

2.0 DRUG HISTORY AND INFORMATION

NDA's: 21-618; 21-681; 21-682
Sequence: 000
Review Number: 1
Submission Type: Original; 505 (b) (2) Designation
Date of Submission: 7/17/03
Information to Sponsor: Yes (x), No ( )

Sponsor: Presutti Laboratories
1607 N. Douglas Ave.
Arlington Heights, IL 60004

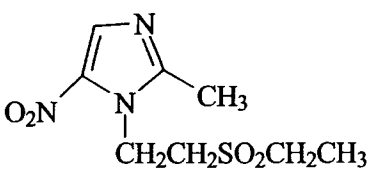
Manufacturer of Drug Substance: [redacted]

Reviewer: Stephen G. Hundley, Ph.D., DABT
Pharmacology/Toxicology Reviewer

Division: Special Pathogen and Immunologic Drug Products
HFD-590

Review Completion Date: 4/2/04

Drug Information
Trade Name: [redacted]
Generic Name: Tinidazole
Chemical Name: 1-[2-(Ethylsulfonyl) ethyl]-2-methyl-5-nitroimidazole
CAS Number: 19387-91-8
Molecular Formula: $C_8H_{13}N_3O_4S$
Molecular Weight: 247.28
Molecular Structure:



Relevant Submission: IND 62,292

Drug Class: Antiprotozoal and Antibacterial 5-Nitroimidazole

Indications: Trichomoniasis (NDA 21-618)
Giardiasis (NDA 21-681)
Amebiasis (NDA 21-682)

Clinical Formulation: Tablets (250 and 500 mg)

Route of Administration: Oral

Proposed Use:

- Trichomoniasis: 1) single 2 g oral dose (one day) with food; 2) [redacted]
- Giardiasis: 1) single 2 g oral dose (one day) with food; 2) [redacted] 3) single 50 mg/kg oral dose to pediatric patients over the age of 3 years (one day) not to exceed 2 g.
- Amebiasis: 1) single 2 g oral dose for 3 consecutive days for intestinal amebiasis; 2) 2 g daily oral dose for 3 to 5 days for amebic liver abscess; 3) single 50 mg/kg oral dose for 3 days to pediatric patients over the age of 3 for intestinal amebiasis not to exceed 2 g daily.

2.1 Study and Literature Articles

The following study was conducted and submitted by the sponsor with the NDA and reviewed.

A Fertility Study of Tinidazole in Male Rats. Study No. [redacted]-453001.

The following literature articles were submitted with the NDA and reviewed.

The Metabolism of Tinidazole in the Rat and Dog. Wood, B.A., et.al., Xenobiotica 3:801-812, 1973.

The Mutagenic Action of Nitroimidazoles. Voogd, C.F., et.al., Mutation Research 66:207-221, 1979.

The Pharmacokinetics, Metabolism, and Tissue Distribution of Tinidazole. Wood, B.A., et.al., J. Antimicrob. Chemother., 10 (Suppl. A):43-57, 1982.

Evaluation of Genetic Damage Induced by a Nitroimidazole Derivative in Human Lymphocytes. Lopez-Nigro, et.al., Toxicol. In Vitro, 15:209-213, 2001.

The following literature articles were previously submitted and reviewed under IND 62,292.

Antitrichomonad Action, Mutagenicity and Reduction of Metronidazole and Other Nitroimidazoles. Lindmark and Muller, Antimicrob. Agents Chemother., 10:476-482, 1976.

Screening for the Mutagenicity of Nitro-group Containing Hypoxic Cell Radiosensitizers using *Salmonella typhimurium* Strains TA 100 and TA 98. Chin, Sheinin, and Rauth, Mutation Res., 58:1-10, 1978.

Structure-cytotoxicity Relationships of Nitroimidazoles in an *in vitro* System. Edwards, Knox, and Knight, Int. J. Rad. Oncol. Biol. Physics, 8:791-793, 1982.

On the Mutagenicity of Nitroimidazoles. Voogd, Mutation Res., 86:243-277, 1981.

Safety Evaluation of Tinidazole, an Antitrichomonal in Rats and Mice. Noguchi, et. al., J. Appl. Pharmacol., 8:1089-1103, 1974.

Effects of Tinidazole on Fetuses and their Postnatal Development in Mice and Rats. Owaki, et.al., J. Appl. Pharmacol., 8:421-427, 1974.

Study of the Effects of Bioshik (Tinidazole) on Fetal Development in Pregnant Rats. Boyadzhieva, Akusherstvo i Ginekologiya, 26:48-51, 1987.

Experimental Study of the Effect of Bioshik (Tinidazole) on some Reproductive Indices. Boyadzhieva, Eksperim. Med i Morf., 29:53-57, 1990.

Sperm Abnormality Assay of Metronidazole and Tinidazole. Pylkkanen and Lahdetie, Mutation Res., 140:137-140, 1984.

Tinidazole (TNZ) (ethyl[2-(2-methyl-5-nitro-1-imidazolyl)ethyl]sulphone) is Mutagenic in a *Salmonella typhimurium* Assay. Coulter and Turner, Mutation Res., 57:97-101, 1978.

Activation of Tinidazole, an Antiprotozoal Drug to a Mutagen by Mammalian Liver S-9. Gupta, Vats, and Juneja, Mutation Res., 370:195-201, 1996.

The Mutagenic Action of Nitroimidazoles. III. Tinidazole, Ipronidazole, Panidazole, and Ornidazole. Voogd, Van Der Stel, and Jacobs, Mutation Res., 48:155-162, 1977.

The Mutagenic Action of Nitroimidazoles. I. Tinidazole, Ipronidazole, Panidazole, and Ornidazole. Voogd, Van Der Stel, and Jacobs, Mutation Res., 26:483-490, 1974.

Mutagenic Activity of 4 Active-principle Forms of Pharmaceutical Drugs: Comparative Study in the *Salmonella typhimurium* Microsome Test and the HGPRT and Na^+/K^+ ATPase Systems in Cultured Mammalian Cells. Dayan, Crajer, and Deguigand, Mutation Res., 102:1-12, 1982.

Mutagenicity of Metronidazole: Structure-Activity Relationships. Rosenkrantz, Speck, and Stambaugh, Mutation Res., 38:203-206, 1976.

Micronucleus Induction in Mouse Bone Marrow Cells of some Nitrofuran, 5-Nitroimidazole, and Nitrothiazole Derivatives used as Trichomonacides in Korea. Paik, Environ. Mutagens & Carcinogens, 5(2):61-72, 1985.

Tinidazole and Emetine Cytogenic Effects Evaluated by the Micronucleus Test in Mice. Leal-Garza, et.al., Arch. Invest. Med., 15:311-316, 1984.

Mutagenic Bioassy of Certain Pharmacological Drugs. III. Metronidazole. Mudry, et.al., Mutation Res., 305:127-132, 1994.

Metronidazole and Misonidazole: Absence of Cytogenic Effects in Euoxic Bacteria and a Mammalian Cell System in vitro. Dunlop, Nitroimidazoles: Chemistry, Pharmacology, and Clinical Application., 171-179, New York, New York, 1982.

3.0 PHARMACOLOGY

3.0.1 Summary

The 5-nitroimidazole class of compounds, including tinidazole and its structural analog metronidazole, exhibit both antiprotozoal and antibacterial activity. These activities and potential mechanisms of action have been established and examined clinically and nonclinically. The Clinical and Microbiology Reviews of the tinidazole application addressed the pharmacology information. The Pharmacology/Toxicology Review and Evaluation, therefore, will not cover the pharmacological activity of tinidazole.

3.1 PHARMACOKINETICS/TOXICOKINETICS

3.1.1 Summary

Tinidazole metabolism studies in rats and dogs indicated that the primary route of excretion was in the urine (60 to 70 percent) with the remainder in feces. Plasma half-lives were approximately 8 to 10 hours in dogs. Over half of the urinary excretion

products from rats and dogs was unchanged tinidazole. Structurally characterized metabolites included ring methyl group hydroxylation and a glucuronide to this hydroxylated product. Tissue distribution data for radioactivity derived from radiolabelled tinidazole indicated no evidence for accumulation in any organ or tissue. Biliary excretion in bile duct cannulated dogs over a 7-hour period represented approximately 2.6 percent of an *iv* dose of radiolabelled tinidazole. Biliary concentrations of radioactivity derived from radiolabelled tinidazole were substantially higher than the plasma levels at the 6- or 7-hour timepoint.

3.1.2 Metabolism, Disposition, & Excretion

The Metabolism of Tinidazole in the Rat and Dog. Wood, B.A., et.al., Xenobiotica 3:801-812, 1973.

The Pharmacokinetics, Metabolism, and Tissue Distribution of Tinidazole. Wood, B.A., et.al., J. Antimicrob. Chemother., 10 (Suppl. A):43-57, 1982.

Metabolism studies were conducted in rats and dogs with [^{35}S]-labelled tinidazole. The urinary elimination of radioactivity derived from 100 and 400 mg/kg oral [^{35}S]-tinidazole doses to rats represented 50 to 64 percent of the original dose while 17 to 25 percent was detected in the feces (5-day cumulative collection of urine and feces). Similar results were obtained following a 130 mg/kg *iv* dose of [^{35}S]-tinidazole. Dogs eliminated 69 to 72 percent of a single oral dose of [^{35}S]-tinidazole in the urine (3 or 100 mg/kg dose levels) over a 5-day post-dosing period. The remainder of the dose was excreted in the feces (approximately 30 percent). In a separate *iv* dosing routine (20 mg/kg) to anesthetized bile duct cannulated dogs, 2.6 percent of the dose was eliminated in bile over a 7-hour post-dosing period. In a separate *iv* dosing portion of the same study, the tinidazole plasma half-life in dogs was approximately 8 hours.

Unchanged tinidazole was the major urinary excretion product for both rats and dogs and represented approximately 57 percent of the urinary radioactivity derived from [^{35}S]-tinidazole. Two urinary metabolites were structurally characterized as 1) the hydroxylated ring methyl derivative of tinidazole, and 2) the O-glucuronide of the ring hydroxymethyl metabolite. Combined, these metabolites represented approximately 24 percent of the urinary radioactivity derived from [^{35}S]-tinidazole. Two additional urinary metabolites were detected but not structurally characterized. Most of the fecal radioactivity from rats and dogs was a combination of unchanged tinidazole and the ring hydroxymethyl tinidazole metabolite.

Tissue distribution of radioactivity in rats dosed *iv* with 12 mg/kg [^{14}C]-tinidazole did not suggest tissue or organ specific retention or accumulation of radioactivity derived from [^{14}C]-tinidazole. Dogs were also dosed *iv* with 12 mg/kg [^{14}C]-tinidazole and tissue/plasma radioactivity ratios generated 6 hours post-dosing did not suggest specific tissue retention or accumulation. However, the bile/plasma radioactivity ratio was approximately 85, indicating substantial biliary elimination following *iv* administration.

3.2 TOXICOLOGY

3.2.1 Nonclinical Toxicology Summary

Single-dose oral toxicity studies with tinidazole generated LD_{50} values of approximately 3,000 mg/kg for rats and 4,000 mg/kg for mice. These values were similar to those reported for metronidazole. The overt clinical signs observed in rats included lethargy, tremor, clonic convulsions and cyanosis which were also the overt clinical signs seen with metronidazole.

The repeat-dose studies cited in the submitted literature included one- and six-month oral dosing (gavage) toxicity studies with tinidazole in Sprague-Dawley derived rats. Tinidazole dose levels in the one-month study ranged from 125 to 4,000 mg/kg. Most of the animals dosed at the 4,000 mg/kg level died by Day 17, which was not unexpected with an LD_{50} value of approximately 3,000 mg/kg. Mortality (2 of 15 females) was also observed at the 2,000 mg/kg dose level. No mortality or clinical signs of toxicity were observed at dose levels of 1,000 mg/kg and lower. No compound-related effects were detected in hematology and serum chemistry at dose levels of 2,000 mg/kg and lower. Gross pathology indicated elevated liver weights and reduced testes and epididymis weights at the 1,000 and 2,000 mg/kg dose levels. Histopathological observations at the 1,000 and 2,000 mg/kg dose levels included pleomorphism in the liver; and atrophy of seminiferous tubules, inhibition of spermatogenesis, and absence of spermatogoniums in the seminiferous tubules of the testes. No compound-related histopathological effects were observed at dose levels of 500 mg/kg and lower. The no observed adverse effect level (NOAEL) for this study was 500 mg/kg.

The six-month oral toxicity study in rats included tinidazole dose levels of 60, 150, 300, and 600 mg/kg. No mortality was observed at any tinidazole dose level and clinical signs of toxicity were not observed. No compound-related effects on hematology and serum chemistry were observed at any tinidazole dose level. Enlarged cecums were observed upon gross pathology at the 300 and 600 mg/kg dose levels. Also observed at the 600 mg/kg dose level were elevated liver weights, reduced testes and epididymis weights, and testicular atrophy. Histopathology of the liver revealed pleomorphism of hepatocytes and slight disarray of hepatic cell clusters at the 300 and 600 mg/kg dose levels. Mild to moderate degeneration of seminiferous tubules in the testes was observed at the 600 mg/kg dose level. The NOAEL for this study was 150 mg/kg.

Reproductive toxicity studies were conducted in rats and mice. Pregnant rats were dosed orally by gavage at tinidazole dose levels of 125, 500, and 2,000 mg/kg on days 9 through 14 of gestation. Pregnant mice were dosed on days 7 through 12 of gestation at tinidazole dose levels of 125, 500, and 2,500 mg/kg. 15 to 17 pregnant dams at each dose level for both rats and mice were sacrificed prior to natural birth for evaluation of fetuses and resorptions. Five pregnant dams at each dose level for rats and mice were allowed to deliver and nurse their offspring; the neonates were monitored over a six-week period for neonatal development. There was no evidence of maternal toxicity to pregnant mice at any dose level. There was also no evidence of fetal toxicity or effects on fetal

development in mice at any of the tinidazole dose levels compared to the zero-level vehicle control. The 2,000 mg/kg tinidazole dose level was maternally toxic to rats with mortality (2 of 15) and lower body weight compared to zero-level vehicle control animals. No evidence of maternal toxicity was reported at the 125 and 500 mg/kg dose levels. Higher incidence of fetal mortality was observed at both the 500 and 2,000 mg/kg dose levels compared to the percent fetal mortality for the zero-level vehicle control (3 vs approximately 8 percent). Skeletal development was affected at the 2,000 mg/kg dose level with absent or rudimentary fifth sternebra (13 percent incidence rate vs 4 percent for the zero-level vehicle control). No skeletal or visceral malformations were observed in fetal rats at any tindazole dose level. The maternal NOAEL for rats was 500 mg/kg while the NOAEL for embryo-fetal toxicity was 125 mg/kg. The maternal and fetal NOAEL for mice was 2,500 mg/kg.

The development phase of this study monitored the maturation and development of neonatal rats and mice for a period of six weeks following birth. Tinidazole dosing to the dams was terminated prior to delivery as noted in the previous paragraph. No effects were observed in neonatal rats and mice at any maternal tinidazole dose level for the following indices: body weight gain, eyelid opening, ear opening, hair growth, incisor appearance, and hearing. All sexual organs were properly developed in both sexes of rats and mice at the six-week post- partum sacrifice.

A separate laboratory evaluated the developmental toxicity of tinidazole in pregnant rats by oral gavage dosing on Days 1 through 21 of gestation at dose levels of 100, 300, and 600 mg/kg. All dams were allowed to give natural birth to their offspring and all offspring were monitored for 2 months following birth. There was no evidence of maternal toxicity at any tinidazole dose level. No skeletal or visceral malformations were observed in the offspring at the 2-month post-partum sacrifice. Fetal toxicity was observed at the 600 mg/kg dose level with a marginally higher incidence of fetal mortality compared to the zero-level vehicle control. The only observed developmental effect was slightly depressed responsiveness in the initial 24 hours after birth for neonates from one dam at the 600 mg/kg dose level. The NOAEL for fetal toxicity was 300 mg/kg. The NOAEL for maternal toxicity was 600 mg/kg.

Reproductive indices were also evaluated in a separate study with male and female rats at oral gavage dose levels of 150 and 300 mg/kg. Sexually immature (1.5 months of age) or sexually mature (3 months of age) males and females were dosed orally by gavage for a period of 20 consecutive days. Males and females were mated for five days following the final dose. Slightly reduced fertility (percent pregnancy) was observed following dosing to sexually immature males or to sexually immature females at the 300 mg/kg dose level (female rats were sacrificed on Day 16 post-mating). A slightly higher percentage of early resorptions was reported for sexually mature females dosed at both the 150 and 300 mg/kg dose levels prior to mating with non-dosed males of the same age. This study suggested that male fertility may have been affected at the 300 mg/kg dose level to sexually immature rats and that 150 mg/kg to sexually mature female rats resulted in embryo toxicity.

The mutagenicity of tinidazole was evaluated by several investigators in the *Salmonella typhimurium* mutagenicity test in the presence and absence of a hepatic S-9 metabolic activation system. Tinidazole was consistently mutagenic in the TA 100 *S. typhimurium* tester strain both with and without the metabolic activation system. Tinidazole generated mixed positive and negative results in the TA 1535, 1537, and 1538 strains and was negative for mutagenicity in the TA 98 strain. Tinidazole was also mutagenic in a tester strain of *Klebsiella pnuemonia.* Tinidazole was negative for mutagenicity in a mammalian cell culture system utilizing Chinese hamster lung V79 cells (HGPRT test system) and negative for genotoxicity in the Chinese hamster ovary (CHO) sister chromatid exchange assay. Tinidazole induced elevated numbers of sister chromatid exhanges in freshly isolated human lymphoctye cell cultures. Tinidazole gave a postive response for *in vivo* genotoxicity in the mouse micronucleus assay with an elevation of micronuclei frequency of approximately 3-fold following *ip* doses of 50 or 100 mg/kg on two consecutive days.

3.2.2 Reproductive and Developmental Toxicology

A Fertility Study of Tinidazole in Male Rats, Study No. [redacted]-453001.

A male rat fertility study was conducted with [redacted]:CD® male and female rats. Male rats received the following oral gavage dose levels of tinidazole for 60 days; 100, 300, and 600 mg/kg/day. Each dose level, including a zero-level vehicle control, consisted of 25 male rats. The tindidazole lot number was 105091 ([redacted] percent analytical purity). An equal number of nondosed female rats at each dose level were used for mating pairs after 30 days of tinidazole dosing to male rats. All animals were sexually mature and randomized by computer for treatment group assignment. The study was conducted for the sponsor by [redacted] in accordance with GLP requirements and was audited by a Quality Assurance group.

Clinical observations of males were made twice daily (once weekly for female rats). The estrous cycle was evaluated prior to mating for all females. Body weights were determined twice weekly for males and for females until gestation. Males and females were paired on study Day 30. Once mating was confirmed the male rats were removed to individual cages and dosing to males was continued to Day 60 of the study. Female weights were determined on Day 0, 3, 7, 10, 13, and 15 of gestation. Food consumption was determined twice weekly for both males and females.

Male and female mating and fertility indices were determined during mating and after mating for evidence of pregnancy. Spontaneous abortions were monitored following Day 1 of gestation through Day 14 of gestation. Pregnancy outcome was assessed on Day 15 of gestation by sacrificing all female rats and determining the number of corpora lutea for each ovary, number of all embryos, total number of implantation sites, early and late resorptions, and embryo viability. A cursory gross pathological exam was also conducted for each female rat on study.

Male rats continued to receive tinidazole dosing until the Day 60 sacrifice. Gross pathological examinations were conducted on each male rat. The following organs and tissues were excised and weighed: brain, epididymis, pituitary gland, seminal vesicle, and testes. Histopathology was conducted on all of the previously listed organs and tissues plus the vas deferens and the coagulating gland of the seminal vesicle. Spermatogenic evaluations were based upon the assessement of sperm morphology and motility from sperm freshly isolated from the epididymis (approximately 200 spermatozoa examined per male). Spermatid count was determined for the testes and epididymis.

Clinical observations indicated red or clear nasal discharge from male rats dosed at the 300 and 600 mg/kg dose levels. Increased salivation was noted until one hour after dosing at the same dose levels. No clinical effects were observed at the 100 mg/kg dose level. Body weight gain was reduced only during study days 0 through 4 at the 600 mg/kg dose level. The mean cumulative body weight gain was also reduced in males at the 600 mg/kg dose level. No body weight gain effects were observed at the 100 and 300 mg/kg dose levels. Food intake was slightly reduced at the 300 and 600 mg/kg dose levels from Day 0 through Day 4 of the study.

Mating indices for males and females were not affected at any tinidazole dose level. Fertility indices, however, were significantly reduced to 45.8 percent in the 600 mg/kg dose group. The fertility index for the zero-level control and 100 mg/kg dose groups was 100 percent. The 300 mg/kg dose group fertility index of 92 percent was not statistically different from the zero-level control group. No gestation effects were noted for gravid female rats at any tinidazole dose level. The following pregnancy effects were noted in gravid females from the 600 mg/kg dose level at the scheduled sacrifice on Day 15 of gestation; decreased number of corpora lutea per dam (13.6 vs 16.6 for the controls), decreased percent of viable embryos per litter (88 percent vs 94.5 percent for the controls), and increased early resorptions (12 percent vs 5.5 percent for controls). No compound related effects were observed in gravid females at the 100 and 300 mg/kg dose levels.

The only gross pathological effects observed in male rats were smaller and softer testes and smaller epididymides at the 600 mg/kg dose level. Testicular weights were reduced by 25 percent in males from the 600 mg/kg dose level compared to the zero-level vehicle control males. Epididymis weights were reduced by 7 and 24 percent in males from the 300 and 600 mg/kg dose levels, respectively, compared to nontreated control males. No gross pathological effects were observed in males from the 100 mg/kg dose level.

The following histopathological effects were observed in the epididymis from males at the 600 mg/kg dose level: moderate to severe hypospermia (16 of 24 males); minimal to mild cellular debris (11 of 24 males); and minimal to mild epithelium vacuolation (2 of 24 males). The following table details the observed testicular histopathology.

Testicular Histopathology

	Zero-Level Control	100 mg/kg Dose Level	300 mg/kg Dose Level	600 mg/kg Dose Level
Spermatid Retention	5 of 25 (Min.)	13 of 25 (Min.)	21 of 25 (Min. & Mild)	16 of 25 (Mild)
Seminiferous Tubule Vacuolation	3 of 25 (Min.)	3 of 25 (Min.)	6 of 25 (Min.)	17 of 25 (Mild to Severe)
Seminiferous Tubule Degeneration	0 of 25	1 of 25 (Mild)	2 of 25 (Min.)	12 of 25 (Moderate to Severe)

The testicular histopathology observed at the 300 and 600 mg/kg dose levels were considered compound related. The histopathology severity classifications were minimal (min.), mild, moderate, and severe. Both the incidence and severity of the histopathology was dose dependent at the 300 and 600 mg/kg dose levels. The testicular effects at the 600 mg/kg dose level were described as partial to complete loss of seminiferous epithelium, degeneration and vacuolation in the seminiferous epithelium, and vacuolation in the basal third of the seminiferous epithelium (Sertoli cell cytoplasm). Retention of spermatids at the 300 and 600 mg/kg dose levels was described as failure of release of step 19 spermatids at stage VIII. Effects observed at the 100 mg/kg dose level were also observed at the zero-level vehicle control and were not considered compound related.

Compound-related spermatogenic effects were observed in the epididymis and testis at the 300 and 600 mg/kg dose levels. Sperm motility was reduced to 66 and 17 percent at the 300 and 600 mg/kg dose levels, respectively (85 percent motility was observed for the nontreated controls). Sperm concentration in the epididymis ([redacted] sperm per gram) was significantly reduced at the 300 and 600 mg/kg dose levels. The epididymis sperm count was reduced by half at the 600 mg/kg dose level. A similar reduction in sperm count in testis was also observed at the 600 mg/kg dose level while effects at the 300 mg/kg dose were not statistically different from nontreated control values for testis sperm count. Statistically significant decreases in normal sperm morphology were observed at both the 300 and 600 mg/kg dose levels (85 and 32 percent normal sperm, respectively). The normal sperm morphology was 99 percent for nontreated control samples. The morphological descriptions included; normally shaped heads that were separated from the flagellum, normal flagellum absent heads, and misshapen heads with normal flagellum.

3.3 CONCLUSIONS AND RECOMMENDATIONS

3.3.1 Conclusions

The male rat fertility study was conducted by the sponsor at the request of the Pharmacology/Toxicology Reviewer in part because of the testicular effects noted in the repeat-dose toxicity studies. Results of the Segment I male rat fertility study confirmed

the testicular toxicity of tinidazole with resulting impaired male fertility at the highest dose tested (600 mg/kg/day). The NOAEL for testicular effects was 100 mg/kg or approximately 0.5-fold the highest human therapeutic dose based upon body surface area conversions. These data suggest that testicular effects due to tinidazole may occur upon prolonged treatment to male patients. All of the primary indications sought by the sponsor are short-term in dosing duration (1 to 7 days) with the exception of [redacted] No additional toxicological concerns were suggested by the nonclinical information for the proposed durations of tinidazole therapy.

3.3.2 Recommendations

The Pharmacology/Toxicology Reviewer recommended that the sponsor conduct a one-month repeat-dose toxicity study in beagle dogs as a post-marketing commitment in order to provide repeat-dose toxicological evaluation in a non-rodent species. Additionally, the repeat-dose study in dogs is needed to determine if a non-rodent species exhibits testicular histopathology similar to the effects observed in male rats.

3.3.3 Suggested Labelling

The following labelling language is proposed by the Reviewer:

Carcinogenesis, Mutagenesis, Impairment of Fertility

[redacted]

Tinidazole was mutagenic in the TA 100, *S. typhimurium* tester strain both with and without the metabolic activation system and was negative for mutagenicity in the TA 98 strain. Mutagenicity results were mixed (positive and negative) in the TA 1535, 1537, and 1538 strains. Tinidazole was also mutagenic in *Klebsiella pnuemonia*. Tinidazole was negative for mutagenicity in a mammalian cell culture system utilizing Chinese hamster lung V79 cells (HGPRT test system) and negative for genotoxicity in the Chinese hamster ovary (CHO) sister chromatid exchange assay. [redacted] Tinidazole was positive for *in vivo* genotoxicity in the mouse micronucleus assay.

Tinidazole reduced fertility and produced testicular histopathology in male rats at a 600 mg/kg/day dose level (approximately 3-fold the highest human therapeutic dose based upon body surface area conversions). Spermatogenic effects resulted from 300 and 600 mg/kg/day dose levels. The NOAEL for testicular and spermatogenic effects was 100 mg/kg/day (approximately 0.5-fold the highest human therapeutic dose based upon body surface area conversions).

Pregnancy: Teratogenic Effects. Pregnancy Category C

The use of tinidazole in pregnant patients has not been studied. Since tinidazole crosses the placental barrier and enters fetal circulation, it should not be administered to pregnant patients in the first trimester.

Embryo-fetal developmental toxicity studies in pregnant mice indicated no embryo-fetal toxicity or malformations at the highest dose level of 2,500 mg/kg (approximately 6.3-fold the highest human therapeutic dose based upon body surface area conversions). In two separate studies with pregnant rats a slightly higher incidence of fetal mortality was observed at maternal dose levels of 500 and 600 mg/kg (2.5- and 3-fold the highest human therapeutic dose based upon body surface area conversions). No biologically relevant neonatal developmental effects were observed in rat neonates following maternal doses as high as 600 mg/kg (3-fold the highest human therapeutic dose based upon body surface area conversions).

Because animal reproduction studies are not always predictive of human response and because there is some evidence of mutagenic potential, the use of tinidazole during pregnancy requires that the potential benefits of the drug be weighed against the possible risks to both the mother and fetus. (See Contraindications).

The italicized text is taken from the sponsor's proposed label and appear to be acceptable to the Reviewer.

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